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LIMITED MULTIPLE-MATING IN MALES AND SINGLE-MATING IN FEMALES OF THE ANT SPECIES, *PARATRECHINA FLAVIPES* (FR. SMITH) (HYMENOPTERA: FORMICIDAE)

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Abstract.—This study determined how many times alates of *Paratrechina flavipes* (Hymenoptera: Formicidae) can copulate in the field and laboratory. In the field, females preferred to mate once and the mating number of males is unknown. In the laboratory, females mated singly but males could inseminate two or three females. The duration of succeeding copulations was greater than the first copulation. Multiple mating males died sooner than single mating ones. The results suggest that male death is promoted by sperm consumption.

Key Words.—Insecta, ant, *Paratrechina flavipes,* mating behavior, Hokkaido Japan

Studies on ant mating behavior have focused on the queen, especially from the social evolution viewpoint (Page & Metcalf 1982, Cole 1983, Crozier & Page 1985). Male mating has been ignored as the female contributes more to the colony throughout her life than the male (Bartz 1982). Other reasons for ignoring male mating behavior include: the amount of sperm is determined when the male ecloses from the pupa (Hölldobler & Bartz 1985), and it is often less than that needed by female ants like *Atta laevigata* (F. Smith) (Corso & Serzedello 1981) or *Solenopsis invicta* Buren (Glancey & Lofgren 1985). Furthermore, observation of single males in nuptial flight is very difficult because of the large number of males present (Talbot 1959, Hölldobler 1976).

The limited amount of sperm in male ants could pose a problem in species whose females mate once. If the female copulates with a mate-experienced male, she may not receive sufficient sperm from him. Sperm scarcity could lead her colony to collapse sooner than the colony of a female who copulated with a "virgin" male. Thus, female rejection of a mate-experienced male could evolve in such an ant species.

There are few social Hymenoptera for which the potential number of male matings is known. *Apis mellifera* L. (Wilson 1971) and *Formica rufa* L. (Marikovsky 1961) are examples of males which mate once. When they terminate copulation their genitalia are destroyed and they soon die. Winged males of *Technomyrmex albipes* Santchi seem to mate once without destruction of their genitalia, but the potential number of matings per male is unknown (Yamauchi et al. 1991). Alates of many ant species, including *T. albipes,* swarm so high in the sky that their mating behavior cannot be seen from the ground. Thus, the possible mating number of males is still unknown.

This paper deals with both field and laboratory observations of the mating

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behavior of the ant, *Paratrechina flavipes* (Fr. Smith) in Tomakomai, northern Japan. I placed special emphasis on determining how many matings alates can conduct.

Materials and Methods

Field Observation.—I visited a broad leaved forest with a 10% mixture of yezo spruces, *Picea jezoensis* Miki, in Tomakomai from 10 to 30 Jun 1986 to observe the nuptial flight of *P. flavipes.* I followed females (n = 1–2 at a time) in flight and recorded the exact time of individual behaviors. I observed 12 females on 25 Jun and seven on 26 Jun; no females were found outside of nests on the other days.

Laboratory Experiments.—In June 1988, I collected female and male alates on nuptial flights, between 09:00 h to 12:00 h (Japan Standard Time, JST), by sweeping vegetation with an insect net. I placed individual alates in 10 or 15 ml vials containing a small piece of moist tissue paper for humidity and took them to the Tomakomai Experiment Forest laboratory. I collected nine females and 20 males on 13 Jun and 61 females and 50 males on 23 Jun. Two females among the nine collected on 13 Jun were excluded from the experiment because they removed their wings after collection.

I randomly selected one male and put it into a transparent polystyrene box (39 × 36 × 14 mm) with plaster of Paris covering the bottom. After the male calmed down, I put one female into the box and observed their mating behavior. After copulation, I replaced her with another female and kept the fertilized female in another box with the same bottom. I repeated this until the male died. I also paired the fertilized female with three unused males to determine whether she would mate again. All the paired alates were selected from the same-day collection.

Results

Field Observation.—I observed completely the copulation of four females on 25 Jun but none on 26 Jun. Three females mated only once but the other mated twice. The copulation durations of single mating females were short, ranging from 49 to 140 sec, while those of the female mating twice were longer: 390 and 731 sec respectively. Copulations of other females, eight on 25 Jun and seven on 26 Jun, were not observed completely and these females shed their wings within 10 min after the copulation and disappeared into the litter layer. Field observations suggest that *P. flavipes* females mate once.

Laboratory Observation.—Six males copulated multiply and they died significantly sooner ($t = 2.78$, $P < 0.02$) than those who copulated once or not at all (Table 1). This suggests a negative correlation between number of male matings and longevity after the last copulation.

There is a significant difference ($t = 2.24$, $P < 0.05$) in the duration of the first mating among these males. Duration of copulation of single mating males is longer than the first copulation of multiple mating males. A two-way ANOVA reveals that in multiple mating males the duration of the first copulation is longer than that of the second one while the duration of copulation is not significantly different among individual males ($Fs = 3.98$, $P > 0.05$) (Table 2). This suggests a positive correlation between the duration and number of copulations.

All females mated in the laboratory experiment were exposed to three additional

Table 1. The number and duration (seconds) of copulations and days from the last copulation to the death for males of *P. flavipes*.

Male	Number	Duration			Days[a]
		First[b]	Second	Third	
m1	3	92	360	205	0.5
m2	2	235	1210	—	1.5
m3	2	60	722	—	0.5
m4	2	287	408	—	1.5
m5	2	40	140	—	1.5
m6	2	128	972	—	0.5
Mean ± SD		140.3 ± 99.5	632.0 ± 410.9	—	1.00 ± 0.50
m7	1	100	—	—	2.5
m8	1	195	—	—	2.0
m9	1	223	—	—	1.0
m10	1	719	—	—	1.0
m11	1	322	—	—	2.0
m12	1	160	—	—	2.0
m13	1	531	—	—	2.0
m14	1	282	—	—	1.5
m15	0	—	—	—	2.0
Mean ± SD		316.5 ± 208.4			1.75 ± 0.50

[a] Significantly different, $t = 2.78$, $P < 0.02$.
[b] Significantly different, $t = 2.24$, $P < 0.05$, using log-transformation.

males after copulation. They did not mate again and eventually shed their wings. They laid eggs in July, which became workers in August, indicating that they were successfully inseminated.

Discussion

The field observations of *P. flavipes* nuptial flights and previous data (Ichinose 1987) indicate that females of this ant mate once. The female that was observed mating twice on 25 Jun 1986 seems to be exceptional. Both of her copulations lasted longer than those of other females. The long durations may be due to her copulating with previously mated males. This inference is supported by the laboratory experiment where the duration of a second or third copulation was significantly longer than those of a first copulation.

Some females used in the laboratory could have mated before collection. This seems improbable, however, because females observed in the field removed their

Table 2. A two-way ANOVA test comparing the duration of first and second copulations in multiple mating males. Variables are transformed logarithmically.

Source of variation	df	SS	MS	*Fs*	Significance level
Mating	1	1.265	1.265	27.978	$P < 0.01$
Individuals	5	0.901	0.180	3.987	ns $P > 0.05$
Error	5	0.226	0.045		
Total	11	2.393			

wings less than 10 min after the end of copulation. Laboratory experiments were started at least 1 h after the collection and all the females had their wings. Multiple mating of male ants is known or suggested to occur in several ant species (Scherba 1961, Kannowski 1963, Ito & Imamura 1974). However, *P. flavipes* is the first species in which the number of male matings has been experimentally shown to be limited, although Hölldobler (1976) has suggested that the number of male matings is limited in *Pogonomyrmex* spp.

Multiple matings in some *P. flavipes* males indicates that their genitalia are not lost at copulation as occurs in *F. rufa* (Marikovsky 1961) or *A. mellifera* (Wilson 1971). The reason that copulation of the male is limited may exist in the amount of their sperm, not in the structure of their genitalia. If *P. flavipes* males cannot produce sperm after eclosion as in other ants (Hölldobler & Bartz 1985), the male should have less sperm at the second copulation than at the first. Thus, the ANOVA test in Table 2 suggests a negative correlation between sperm amount and copulation duration in the males. If so, the sperm amount in the male decreases in the order: the multiple mating male at the first copulation; the single mating male; and the multiple mating male at the second copulation. Because survival after the last copulation is shorter in multiple mating males than in single mating ones, sperm usage may promote the death of *P. flavipes* males.

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